IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Gregory T. Bleck, et al.
Serial No.: 10/759,315 Group No.: 1633
Filed: 1/16/04 Examiner: Riggins

Entitled: **PRODUCTION OF HOST CELLS CONTAINING MULTIPLE INTEGRATING VECTORS BY SERIAL TRANSDUCTION**

THIRD DECLARATION OF DR. GREGORY BLECK

I, Dr. Gregory Bleck, state as follows:

1. My present position is Senior Director, Cell Line Engineering Gala Biotech, a Subsidiary of Catalent Pharma Solutions Company.

2. I am an inventor of the above referenced patent application.

3. It is my understanding that the Examiner has argued that one of skill in the art would have known to increase the production of a desired protein by increasing the number of viral integrations and would have known to increase the number of viral integrations by increasing the MOI.

4. In my previous Declaration, I established that references such as Arai et al., Virology 260:109-115 (1999) and Coffin et al., Development and Applications of Retroviral Vectors, Chapter 9 in Retroviruses, 1997, p. 437-473, specifically teach away from the current claims and that one of skill in the art would be "discouraged" from using the claimed multiplicity of infection and copy insert number to obtain cells for the production of a secreted protein. However, the Examiner has attempted to rebut these arguments. As I demonstrate below: 1) a person of ordinary skill in the art such as myself would not interpret Arai et al. and Coffin et al., as the Examiner has; 2) the newly cited Kustikova and Zielske references are not prior art to the present claims and do not support the Examiner's arguments; and 3) prior to the present application and its parent applications, persons of skill in the art were discouraged from

introduction of multiple copies of retroviral vectors into immortalized mammalian cells because they thought that methylation and/or gene interference would decrease protein production.

4. At pages 13-14 of the Office Action, the Examiner addresses my previous Declaration with respect to Arai et. al. and Coffin et al. The With respect to Arai et al., the Examiner states:

> Arai et al. teach that the number of proviral integrations (and therefore, protein production) can be increased by increasing MOI (p. 112, column 1, Fig. 3) and that 15 integrations can be obtained with an MOI of 30. Although they teach that proviral integration with a very high copy number seems to cause cell death, Arai et al. do teach that not cells [sic] are dying and therefore, one of skill in the art would have known to use routine experimentation to clone the viable cells that contain a very high number of integration events and produce cell lines that synthesize high amounts of recombinant protein. Therefore, the art does not teach away from the claim invention. . . . One of skill in the art would use only routine experimentation to optimize the results, and by doing this one of skill in the art would have necessarily obtained integrations within the broad range of 10 to 100."

The Examiner makes several assumptions here that are either scientifically incorrect or that have a better scientific explanation. First, the examiner assumes that the cells that do not die have a very high copy number of integrated vectors. There is no evidence for this. Arai did not clone or determine the number of integrated vectors in those cells. Second, a person of ordinary skill in the art would expect that the surviving cells did not have high numbers of integrations. Retroviral vectors do not transduce cells that are not dividing. The most reasonable explanation is that the surviving cells were not undergoing cell division during the transduction period and thus did not become transduced or that the cells were at a point in the cell cycle so that they were not exposed to vector at the optimum time period for transduction and thus only had low numbers of integrations. A person of skill in the art would believe that this is a valid explanation, especially when Arai teaches that the major factor for apoptosis was probably a high number of integrations and insertional mutagensis.

5. With respect to Coffin et al., the Examiner states:

> Regarding the argument that Coffin teaches that insertional mutagenesis by retroviral vectors in the instant case, because the claims encompass a host cell in vitro and the combined teachings of Mathor et al. and Felts et al. are drawn to the in vitro production recombinant proteins. Applicant's argument that the teachings of high numbers of integrations and insertional mutagenesis would apply both in vivo and in vitro is not

found persuasive because Coffin et al. refers to gene therapy in humans where malignant transformation can endanger the patient life, which cannot be compared to a cell in culture, wherein malignant transformation does not endanger anybody's life and does not impede the cell from producing the protein of interest (also see below).

Here, the examiner is missing the point. Coffin et al. confirms the teaching of Arai et al. that the incorrect use of retroviral vectors can lead to insertional mutagenesis. Furthermore, the Examiner's assumption that malignant transformation or other mutagenesis would not impede an immortalized mammalian cell from producing a protein of interest has no scientific basis. In fact, if an immortalized mammalian cell is mutagenized or transformed in some way by the vector, it is almost certain that production of the desired protein would be affected. The recombinant protein production industry relies on the use of standardized immortalized mammalian cells whose growth is predictable. Cells with additional mutations would be highly undesirable.

6. At page 16 of the office action, the Examiner argues that art "clearly demonstrates that one of skill in the art would not have been discouraged by the teachings of Arai et al. and coffin et al." The Examiner goes on to make arguments based on Kustikova et al., Blood 102:3934-37 (2003) and Zielske et al., Mol. Therapy 9:923-31 (2004). It is my understanding that the filing date of the present application is January 16, 2004 and that it is a continuation-in-part of a parent application filed June 29, 2001. The parent application, which has issued as U.S. Pat. No. 6,852,510, discloses the number of integrations in the current claims as well as a serial transduction process. See, e.g., Column 2, lines 28-35; Column 32, lines 1-5; and Column 33, lines 42-60. Thus, Kustikova and Zielske were published well after the disclosure in my original patent application. Contrary to the Examiner's assertions, these references confirm to a person of skill the art the uncertainty that was associated with introducing multiple copies of a retroviral vector into a host cell.

7. Kustikova directly addresses the fact that insertional mutagenesis is a problem when using retroviral vectors and then goes on to describe methods for finding the correct dose to use. If those of skill in the art believed that routine experimentation could be used to make cell lines with the claimed number of integrations, then there would have been no need to do the experiments described by Kustikova and those experiments would not have been worthy of

publication. What Kustikova actually shows is that those of skill in the art would have been discouraged from intentionally making cell lines with high numbers of retroviral integration because of insertional mutagenesis. Kustikova actually confirms my previous statements and underscores the fact the producing viable cells with high numbers of retroviral integrations such as those claimed was unpredictable to those of skill in the art and needed to be investigated.

8. Zielske also confirms my previous statements. The same arguments apply as with respect to Kustikova. Those of skill in the art actually saw a need to investigate the correlation between MOI, insert copy number and viability. Again, this underscores the fact that those of skill in the art did not believe that routine experimentation could be used to produce the claimed cell lines or methods. Furthermore, Zielske found that transgene expression reach a plateau after four integrations. This further teaches away from the claimed methods – if only four integrations are needed for maximum expression, why introduce more? As shown in the next paragraphs, those of skill in the art believed that expression reached a plateau because of viral interference, gene silencing or methylation.

9. Walker et al., Walker, Human Gene Therapy. Jun 1996, Vol. 7, No. 9: 1131-1138 (Tab 1), teaches that: "Simultaneous retroviral transductions were infrequent events. In addition, transduction of previously infected cells (sequential transductions) occurred at lower than expected frequencies. Our data suggest that there is quantifiable viral interference in sequential retroviral transductions. This interference occurs by a mechanism that appears to be independent of the amphotropic retroviral receptor." Likewise, Bestor, J. Clin. Invest., 105(4):409-411 (2000) teaches retroviruses and repeated genes are often silenced or suppressed by mammalian cells. Because of viral interference and gene silencing or suppression, a person of ordinary skill in the art would be discouraged from using sequential transductions to increase viral insert number and would be discouraged from attempting to create immortalized mammalian cell lines with the claimed number of insertions.

10. In the Interview on April 24, 2008, the Examiner and the Examiner's supervisor indicated that the arguments above would be more persuasive if the claims were limited to immortalized mammalian cells. It is my understanding that the claims have been amended to

reflect this. Further, the Examiners indicated that the teachings of the gene therapy papers we discussed in the interview such as those described above did not necessarily apply to immortalized mammalian cell cultures. I disagree with this because the prior art concern with viral interference, gene suppression and gene silencing would apply regardless of the situation was gene therapy or making immortalized mammalian cells to produce a protein of interest. Nevertheless, I note if it is the Examiner's position that those references only apply to gene therapy situations, then gene therapy prior art references such as Mathor et al., which addresses the use of normal human keratinocytes for gene therapy, Felts et al., which addresses "fields for which highly efficient gene delivery is essential" and refers specifically to gene therapy, and Inaba et al., which addresses the use of endothelial cells for gene therapy (as well as Kustikova and Zielske), do not apply to the use of retroviral vectors in immortalized mammalian cells such as those exemplified throughout the patent application.

11. In the Interview, we also discussed Table 1 at page 10373 of Mathor et al. The Examiners indicated that this Table shows that increasing the number of integrations increases protein production. A person of ordinary skill in the art would not interpret the data in that manner. The data shows that at 8 integrations, 1140 ng/10^6 cells/day of protein is produced, and that when 15 integrations were obtained, the protein production decreased to 1014 ng/10^6 cells/day or protein produced. This indicates that protein production had reached a plateau and that further introduction of retroviral vectors did no good or decreased protein production. Thus, one of skill in the art would conclude from the data additional integration past 8 integrations were not needed or not desirable.

12. I further declare that all statement made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: May 28, 2008

/Gregory Bleck/
Dr. Gregory Bleck